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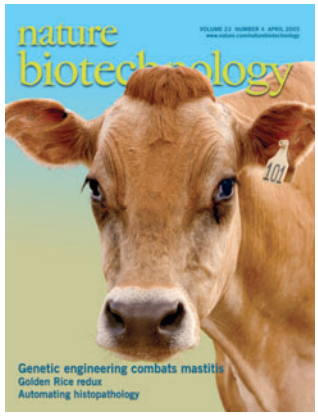
Regulatory regimes for transgenic crops

To the editor:

In presenting their justifications for reducing the regulatory burden on transgenic food crops (*Nat. Biotechnol.* **23**, 439–444, 2005), we feel that Strauss and colleagues significantly misrepresent the implications and rationale of our report *Genome Scrambling-Myth or Reality? Transformation-Induced Mutations in Transgenic Crop Plants*[1]. Unlike their characterization of our work, we did not specifically "argue for rejection if even a single base pair is changed." In full, our relevant recommendations were that "transgenic lines containing genomic alterations at the site of transgene insertions be rejected" and that "the insertion of superfluous DNA be considered unacceptable."

Leaving aside the fact that a single base pair change may result in serious phenotypic consequences, these recommendations are best viewed in context. As documented in the report, thorough analysis reveals that all particle bombardment transgene insertion events include extensive rearrangements or loss of host DNA as well as insertion of superfluous DNA. Furthermore, a large fraction of even apparently simple *Agrobacterium tumefaciens*–mediated transgene insertion events also result in large-scale host DNA rearrangement or deletion and superfluous DNA insertion[2]. For example, loss of 76 kbp of host DNA[3] and duplication/translocation of up to 40 kbp of host DNA have been reported at T-DNA insertion sites[4].



Widespread use of transgenic crops carrying insertion-site mutations of this magnitude will, in our opinion, lead sooner or later to harmful consequences. Nevertheless, detailed inspection has shown that mutations such as these would almost certainly pass unnoticed through both the molecular and phenotypic characterization stages of the regulatory systems of both the European Union and the United States[5–8].

We do agree with Strauss and colleagues that analysis of the phenotype is the one true measure of safety. However, rigorous assessment only at the phenotypic level is time consuming, expensive and, more importantly, of unproven effectiveness[9]. In this context, our recommendations for the detection and elimination of transformation-induced mutations from commercial crop plants are conceived as a straightforward and effective way to reduce the probability of unexpected deleterious phenotypes arising in transgenic crop plants and of protecting consumers and others from an unnecessary risk.

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To the editor:

In the April issue (*Nat. Biotechnol.* **23**, 439–444, 2005), Strauss and colleagues argue that the methods used to produce food crops should not be the focus of regulatory oversight, only the phenotypic traits of the resultant plants as defined in terms of standard agricultural practice. They propose that any risk and safety assessments of crops produced by genetic engineering (GE) should be based only upon the nature of the introduced genes. They also claim that transgenic crops face a "daunting" array of regulatory requirements. However, safety testing requirements in the United States are largely voluntary and in my view inadequate (for a review of regulations from my perspective, see ref. 1). Safety concerns related to the GE process itself as well as its unintended consequences are set aside by Strauss and colleagues as irrelevant, for they claim that the products of genetic events that occur naturally and with standard plant breeding techniques are fundamentally the same as those that occur with GE. Are these arguments a valid reflection of what is known about the precision and consequences of the GE process compared with naturally occurring genomic variation?

The basic assumption underlying the concept of a one-to-one relationship between the transgene and the resultant phenotype is that the GE process is relatively precise. However, none of the current transgene insertion techniques permits control over the location of the insertion site or the number and orientation of the genes inserted. Indeed, over one-third of all *Agrobacterium tumefaciens*–mediated insertion events disrupt functional DNA[2,3]. These and related transformation and cell culture–induced changes in chromosomal structure have been recently documented in great detail[4]. For example, translocations of up to 40 kb[5], scrambling of transgene and genomic DNA[6], large-scale deletions of over a dozen genes[7] and frequent random insertions of plasmid DNA[8] can all be caused by the procedures used to make transgenic plants. In fact, the most commonly used transformation procedure is sometimes itself

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used as a mutagen[9] and can activate dormant retrotransposons that are mutagenic[10]. Moreover, mutations linked to the transgene insertion site cannot be removed by additional breeding as long as there is selection for the transgene itself. Collectively, these data indicate that the GE process itself is highly mutagenic.

Some modern breeding technologies introduce new traits into plants via chemical or radiation mutagenesis or by wide cross-hybridizations that overcome natural species barriers. Mutagenesis was used in the United States during the middle part of the past century, but food crops made by this technique now constitute less than a few percent of US production, with sunflowers being the major representative[11]. However, plants produced by wide crosses, such as those between quackgrass and bread wheat to yield a widely planted grain that has all of the chromosomes of wheat and an extra half genome of the quackgrass, although unique, are fundamentally different from those produced by either mutagenesis or GE. In wide crosses and other forms of ploidy manipulation, there are clearly changes in gene dosage, and proteins unique to only one parent can be produced in the hybrid, but there is no a priori reason to assume that mutations are going to occur simply because there is a change in chromosome or gene number. Although the extent and suddenness of all of these modern breeding technologies are unlike anything known to occur during the course of evolution or with traditional breeding, only GE and mutagenesis introduce large numbers of mutations. Any new cultivars derived by the latter two methods should be subjected to similar regulatory requirements.

Strauss and colleagues correctly state that plants normally contain the same *A. tumefaciens* and viral DNA sequences that are used to create GE transfection constructs, but fail to point out that with GE these pieces of DNA are part of a cassette of genes for drug resistance, commonly along with strong constitutive viral promoters (e.g., cauliflower mosaic virus promoter), which are used to express foreign proteins at high levels in all parts of the plant—hardly a natural event. They incorrectly imply that changes in ploidy, gene copy number, recombination and high genomic densities of transposable elements in normal plants continually lead to mutations and changes in gene expression similar to those caused by GE.

Ploidy is notoriously unstable in plants, but changes involve moving around large blocks of intact genes while maintaining their regulated expression pattern. It should also be remembered that recombination is not the same as random mutagenesis, for there has been tremendous selective pressure for alleles to express functionally similar proteins. The statement that "retrotransposons continuously insert themselves between genes" is incorrect, for these high-copy number elements are very rarely transpositionally active in normal modern food plants[12], have evolved and rearranged in the distant past[13], but can be activated by tissue culture or by mutagenesis[10]. In fact, their discovery by Barbara McClintock was facilitated by the use of mutagenized corn[12].

In contrast to Strauss and colleagues' proposal that regulatory efforts should focus on the expression of the transgene, I believe that the potential negative impact on nutritional content or increase in dangerous metabolites are the major hazards associated with highly mutagenic plant transformation techniques. Although it is widely recognized that the breeding of some crops can produce varieties with harmful characteristics, millennia of experience have identified these crops, and breeders test new cultivars for known harmful compounds, such as alkaloids in potatoes[14,15]. In contrast, unintended consequences arising from the random and extensive mutagenesis caused by GE techniques opens far wider possibilities of producing novel, toxic or mutagenic compounds in all sorts of crops. Unlike animals, plants accumulate thousands of nonessential small molecules that provide adaptive benefits under conditions of environmental or predator-based stress[16]. Estimates are that they can make between 90,000 and 200,000 phytochemicals with up to 5,000 in one species[17]. These compounds are frequently made by enzymes with low substrate specificity[18] in which mutations can readily alter substrate preference[19,20].

There are many examples of unpredictable alterations in small-molecule metabolism in transgenic organisms. In a yeast strain genetically engineered to increase glucose metabolism, the transformation event caused the unintended accumulation of a highly toxic and mutagenic 2-oxoaldehyde called methylglyoxal[21]. In a study of just 88 metabolites in three groups of potatoes transformed with genes for bacterial and yeast enzymes that alter sucrose metabolism. Roessner *et al.*[17] found that the amounts of the majority of these metabolites were significantly altered relative to controls. In addition, nine of the metabolites detected in these transgenic potatoes were not detected in conventional potatoes. Given the enormous pool of plant metabolites, the observation that 10% of those assayed are new in one set of transfections strongly suggests that undesirable or harmful metabolites may be produced and accumulate[22]. Contrary to the suggestions of Strauss and colleagues, Kuiper *et al.*[23] strongly recommend that each transformation event should be assayed for these types of unintended events by metabolic profiling.

A well-documented horticultural example of unintended effects is the alteration in the shikimic acid pathway in *Bacillus thuringiensis* (*Bt*) toxin corn hybrids derived from Monsanto's MON810 and Syngenta's *Bt*11 plants as well as glyphosate-tolerant soybeans. Stem tissue of both groups of plants has elevated levels of lignin, an abundant nondigestible woody component that makes the plants less nutritious for animal feed[24,25]. Components of this same biochemical pathway also produce both flavonoids and isoflavonoids that have a high nutritional value, and rotenone, a plant-produced insecticide that has been associated with Parkinson disease[26]. Isoflavonoids are abundant in legumes like soy beans, and rotenone is synthesized directly from isoflavones in many legume species[27]. Because of the promiscuity of many plant enzymes and the large and varied substrate pools of phytochemical intermediates, it is impossible to predict the products of enzymes or regulatory genes mutated during the transformation event[22]. Although I am not aware of any testing of GE soybeans for rotenone, it has been shown that glyphosate-tolerant soybeans sprayed with glyphosate have a reduced flavonoid content[28].

The safety testing of GE crops need not be as extensive as that done with drugs, food additives or cosmetics. Many suggestions have been put forward (e.g., see refs. 1,4,23,29) including those by the World Health Organization[30]. I believe that the most important safety tests include metabolic profiling to detect unexpected changes in small-molecule metabolism[23] and the Ames test to detect mutagens[31]. Molecular analysis of the gene insertion sites and transformation-induced mutations[4] should also be performed along with both multigenerational feeding trials in rodents to assay for teratogenic effects and developmental problems, and allergenicity testing performed according to a single rigorous protocol[30]. The animal studies are of particular importance for crops engineered to produce precursors to highly biologically active compounds, such as vitamin A and retinoic acid, molecules that can act as teratogens at high doses[32].

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In summary, Strauss and colleagues state that there is a low risk from the consumption of transgenic plants "where no novel biochemical or enzymatic functions are imparted." The question is, of course, how can one know if a novel and potentially harmful molecule has been created unless the testing has been done? How can one predict the risk in the absence of an assay? Because of the high mutagenicity of the transformation procedures used in GE, the assumptions made by Strauss and colleagues and by the US Food and Drug Administration[33] about the precision and specificity of plant genetic engineering are incorrect. Nonetheless, it appears that the position of Strauss and colleagues and the agbiotech industry, as well as the current US regulatory framework for the labeling and safety testing of transgenic food crops, is to maintain the status quo and hope for the best.

The problem is that there are no mandatory safety testing requirements for unintended effects[1] and that it may take many years before any symptoms of a disease arising from a transgenic product to appear. In the absence of strong epidemiology or clinical trials, any health problem associated with an illness caused by a transgenic food is going to be very difficult, if not impossible, to detect unless it is a disease that is unique or normally very rare. Therefore, although GE may enhance world health and food crop production, its full potential may remain unfulfilled unless rigorous prerelease safety testing can provide some assurance to consumers that the products of this new technology are safe to eat.

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Strauss and colleagues respond: Wilson *et al.* claim on the one hand that their report "did not specifically 'argue for rejection if even a single base pair is changed,'" while recommending that "transgenic lines containing genomic alterations at the site of transgene insertion be rejected." In addition, in their original report, they further state that they "recommend that both the transgene insertion event (including all transferred DNA and a large stretch of flanking DNA) and the original target site be sequenced and compared as the only known way to definitively determine whether gene sequences have been disrupted." In the context of their discussion, even a single base pair change is clearly considered to be a "genomic alteration," so we believe that we have accurately represented the implications and rationale of their position.

Regarding the possibility that some genomic changes occur due to transformation, we never denied that this occurs, and in fact cited their study as a source for our statement that "unknown mutations and chromosomal translocations can occur during the transformation and regeneration process." Where we differ with Wilson *et al.* is in their opinion that such mutations will "lead sooner or later to harmful consequences." There is no documentation of such harmful consequences in their report for products that have undergone phenotypic screening for commercial release.

A central point of our Perspective was that a very large number of genomic and gene differences already exist within crop cultivars, and even among individual plants within a cultivar, without producing any harmful consequences (for another striking example, see ref. 1). Thus, the assumption of the inevitability of harmful consequences from genomic differences associated with gene transfer ignores the ubiquity of extensive genome sequence variation within existing food crops.

Although Wilson *et al.* agree with us that "analysis of the phenotype is the one true measure of safety," they nonetheless state that phenotypic analysis is of "unproven effectiveness" and suggest that genomic sequence data would be more reliable or effective. Both of these arguments are flawed. First, phenotypic analysis has been extremely effective in the development of many thousands of commercial cultivars in a wide range of crops for several generations. Second, how Wilson *et al.* propose to distinguish the toxicologically silent genomic differences that are abundant in crop plants from ones that might actually have phenotypic consequences is addressed neither in their original report nor in their comment.

In his letter, Schubert raises several issues, many of which have been addressed extensively in published literature. For completeness, we address these issues here in summary fashion:

Alleged lack of precision in genetic engineering (GE). The lack of precision due to random gene insertion and genomic alteration is often raised as a criticism of GE. However, conventional breeding is based on essentially random induction or assembly of mutations, followed by selection among a multitude of unpredictable and often imprecise natural recombinations between